Sexual biology of *Pandemis pyrusana* (Lepidoptera: Tortricidae) under laboratory conditions

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ABSTRACT

Laboratory studies were conducted to characterize some aspects of the sexual biology of *Pandemis pyrusana* Kearfott. Both males and females were sexually active during their first scotophase. Virgin females held at 22°C started calling the first night 4 - 5 hrs into scotophase. Calling by virgin females occurred earlier and continued longer into scotophase after the first night. Mating lasted 3 - 4 hrs and both sexes mated only once per evening. Calling frequency by mated females was lower than for virgins and dropped off sharply after 2 nights. Forty percent of females mated more than once during the 6-day test. Males mated on consecutive scotophases, but the percentage of subsequent copulations passing a spermatophore declined with age. Oviposition occurred throughout a diurnal cycle, but was concentrated during early scotophase. Females laid an average of four egg masses from which 219 larvae eclosed. Egg mass size, number of larvae emerging, and the number of larvae emerging per egg mass area declined with subsequent egg masses.

Key words: *Pandemis*, leafrollers, apple, sexual behaviour

The pest status of the tortricid moth, *Pandemis pyrusana* Kearfott (PLR) in apple orchards in Washington has increased during the past 15 years (Brunner 1983), especially following the adoption of mating disruption for codling moth, *Cydia pomonella* (L.) and the resultant decrease in use of the broad spectrum organophosphate insecticides (Brunner *et al.* 1994; Knight 1995). PLR have two generations in central WA and overwinters as diapausing second- and third-instar larvae in bark crevices (Brunner and Beers 1996). The first flight begins in late May and the second flight peaks in late August. Control of PLR in tree fruit production has typically been with organophosphate insecticides, although the use of *Bacillus thuringiensis* Berliner has increased recently (Washington Department of Agriculture 1995, Knight 1997). To conserve biological control agents of secondary pests, such as leafhoppers, aphids, and leafminers in orchards treated with sex pheromones for mating disruption of codling moth (Knight 1995), similar non-disruptive approaches like mating disruption are needed for PLR and other leafroller species (Alway 1996).

Knowledge of the sexual behaviour of PLR is a prerequisite for development of mating disruption as an effective management tactic (McNeil 1991). The sex pheromone of PLR is a 94:6 blend of (Z)-11-tetradecenyl acetate and (Z)-9-tetradecenyl acetate (Roelofs *et al.* 1977). Traps baited with sex pheromone lures are used to monitor populations of PLR (Madsen *et al.* 1984) and to time insecticide sprays (Brunner and Beers 1996). Knight *et al.* (1994) used a pheromone-baited timing trap in the field and an ultrasound motion detector in the laboratory to determine the circadian periodicity of PLR moth activity. Other important aspects of PLR adult sexual behavioural ecology, such as temporal patterns of calling, mating, and oviposition and the influence of mating status on these behaviours have not been reported. This paper reports results from laboratory studies with PLR to characterize these aspects of its sexual behaviour.

MATERIALS AND METHODS

A laboratory colony of PLR was established with larvae collected from several apple orchards in Yakima County, WA in 1992, and larvae have been added to the colony from additional

orchards each year. Larvae were reared on a synthetic pinto bean diet (Shorey and Hale 1965) at 24°C and a 16:8 (L:D) photoperiod in 30 ml plastic cups. Adults were supplied with a cotton wick saturated with a 10% honey solution. Adult sexual behaviours were studied at 22 ± 2°C, 50 - 65% relative humidity, and a 16:8 (L:D) photoperiod. A reversed photoperiod was used to facilitate observations of moth behaviour (lights on at 0900 hr and off at 1700 hrs). Light levels were controlled by time clocks which switched off and on a series of incandescent light sources during the 60 min dusk (0800-0900 h) and sunrise (1700-1800 h) periods (Knight *et al.* 1994). Illumination during scotophase was provided by a light covered with a red acetate filter.

Calling behaviour. The effect of mating on female calling behaviour was studied by recording the occurrence of calling of 50 newly-mated and 50 virgin females for 30 sec every 30 min for 5 and 6 nights, respectively. Presumed mated females were dissected at the end of the test to confirm their mating status and only the data for mated females were used. All female moths were < 24h-old at the start of the experiment. Moths to be mated were placed with a virgin 2-day-old male for 24 h. Observations of female calling behaviour were made for moths kept in 250 ml waxed paper cups covered with a clear polyethylene film. Calling behaviour was characterized by wing elevation (up to 45°) and a downward extension of the abdomen. Calling frequency (the number of 30-min intervals during which calling was observed in each scotophase) for mated and virgin females was transformed (square root [x + 0.01]) and subjected to analysis of variance (ANOVA) using age as a repeated measure (SAS Institute 1985). If the age-by-mating-status interaction proved to be significant, a one-way ANOVA was used to compare treatments (mating status) for each age separately.

Mating behaviour. To test whether males and females mated more than once, 80 virgin pairs (< 24 h-old) were placed in cups during photophase, and males were replaced with a virgin male (< 48 h-old) in half of the cups and females were replaced with virgin females (< 24 h-old) in the other half each day for 6 days. Moths were observed every 30 min during scotophase to determine their mating status. Females from both sets of cups were dissected after the test to determine whether one or more spermatophores had been passed. Previous dissections of 500 females following a single mating episode did not find more than 1 spermatophore (unpubl. data). Therefore, the number of spermatophores dissected from a female was considered to be equivalent to the number of copulations. The success of males passing a spermatophore as a function of age (number of previous matings) was analyzed with regression analysis using the data from the second set of cups.

Oviposition. Oviposition was studied by pairing 30 virgin male and females for 24 h in cups. Egg masses were collected sequentially and placed in cups until egg hatch was completed. The number of larvae eclosing from each egg mass was counted and the area of each egg mass was measured with a LI-3000 portable area meter (LI-COR, Lincoln, NE). Regressions were fitted to determine the relationship between egg mass sequence (i.e., number of egg masses previously deposited by a female) and egg mass area, numbers of larvae hatching, and numbers of larvae hatching per egg mass area. Temporal patterns of oviposition on a wax paper substrate were measured for 50 mated females using a clock-driven rotating oviposition apparatus at 20°C and a 16:8 (L:D) with lights-off at 2200 h (Knight 1996). Newly emerged females were placed in cups with a male (< 24 h-old) for 24 h and females were transferred to the apparatus between 1000-1100 hours. Oviposition was measured for 4 nights.

RESULTS

Calling behaviour. Female calling occurred throughout the entire scotophase. However, virgin females started calling during the first evening on average 4.5 h into scotophase (Fig. 1a, 1b) and continued to call for 1- 2 h. Both the start and the duration of calling by virgin moths was significantly affected by moth age: $F = 5.2$; df = 5, 235; $P < 0.001$ and $F = 7.0$; df = 5, 258; $P <$

0.0001, respectively. Older virgin moths started calling earlier (Fig. 1a) and called longer (Fig. 1b) than younger virgin moths. No differences in calling were found for moths 2 - 6 d-old. Initiation of calling during scotophase was not different between mated and virgin females (F =

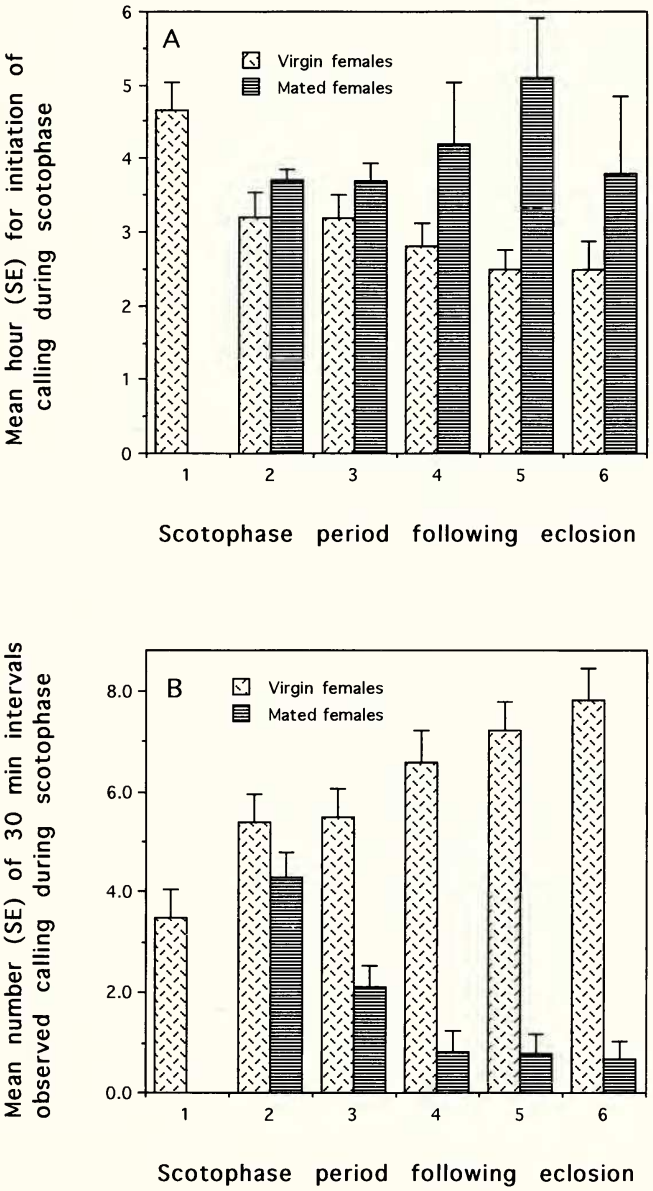

Figure 1. Mean hour during an 8-h scotophase that virgin and mated *Pandemis pyrusana* females initiated calling (A) and the mean number of 30 min intervals during scotophase that virgin and mated females were observed calling during a 30 sec observational period (B). Data were collected for 6 consecutive nights after eclosion. Mated females were mated during the first scotophase period and data are shown for periods 2 - 6 only. Error bars are mean standard errors.

2.72; df = 1, 32; $P = 0.11$), and did not vary with respect to age ($F = 0.38$; df = 4, 128; $P = 0.82$)(Fig. 1a.). However, the frequency of calling by mated females was significantly lower than for virgin moths across the five nights ($F = 175.9$; df = 1,70; $P < 0.001$). Calling by mated females was significantly affected by age ($F = 13.6$; df = 4, 135; $P < 0.001$). Mated females significantly decreased their calling on each of the first 2 nights after mating and then called infrequently for the last 3 nights of the test (Fig. 1b).

Mating behaviour. Eighty-six percent of females mated when paired with a male moth for 24 h. Forty-four percent of these females mated more than once during the 6 nights when presented with a new virgin male each scotophase. However, only 64% of the females observed mating more than once contained two spermatophores. Females had a mean refractory period of 2.2 ± 0.3 days before mating again. Mating began a mean (± SE) of 4.1 ± 0.3 h into scotophase and lasted for 4.2 ± 0.3 h. Thus, both males and females mated only once during the 8 h scotophase. A high percentage of moths were *in copula* at the end of scotophase. Following lights-on, these pairings generally ended. Eighty-three percent of the males mated on each of the 6 nights when paired with a virgin female. However, the proportion of male moths successfully passing a spermatophore during copulation (**y**) was significantly affected by the number of previous matings (**x**):

Equation 1: $\mathbf{y = 1.42 - 0.61x + 0.08x^2}$ ($R^2 = 0.99$, $P = 0.01$).

Oviposition. Egg masses were typically laid within 24 h after mating occurred. Females laid (one to six) egg masses and averaged 4.1 ± 0.2 egg masses over the 5-day test. The mean number of eggs hatching per female was 219.0 ± 12.2. Nearly 90% of egg masses were laid during scotophase with the mean time of oviposition occurring at 1.9 ± 0.3 h into scotophase. The order of egg mass deposition negatively affected ($P < 0.05$) the mean egg mass size, the number of larvae that successfully eclosed, and the number of larvae eclosing per egg mass area (Table 1).

Table 1

Regression analysis of mean egg mass size (mm^2), the number of larvae eclosing, and the hatching rate (larvae per mm^2) as a function of egg mass order for *Pandemis pyrusana* under laboratory conditions at 22°C, 16:8 L:D.

Egg mass order	No. of egg masses	Mean area (SE) of egg mass (mm^2)	Mean no. of larvae hatching	Mean no. of larvae per mm^2
1	27	13.8 (0.8)	102.4 (5.9)	7.5 (0.2)
2	26	9.7 (0.8)	78.0 (6.5)	7.9 (0.4)
3	24	8.0 (0.6)	32.3 (7.2)	3.7 (0.8)
4	21	5.7 (0.6)	11.8 (6.1)	1.8 (0.8)
5	11	4.3 (0.7)	10.9 (7.4)	3.7 (1.3)
6	2	2.3 (0.7)	0.0 (0.0)	0.0 (0.0)
Slope (SE)		-2.37 (0.25)	-25.97 (3.67)	-1.72 (0.22)
P-value		< 0.001	<0.001	<0.001
R^2		0.45	0.55	0.36

DISCUSSION

During the past 8 years we have been developing the use of sex-pheromone-based mating disruption as a selective, non-insecticidal approach to manage a suite of tortricid species injurious to tree fruits in the western USA, such as codling moth (Knight 1995), orange tortrix,

Argyrotaenia citrana (Fernald), (Knight 1996), obliquebanded leafroller, *Choristoneura rosaceana* Harris, (Knight *et al.* 1998), and PLR. Successful incorporation of mating disruption technology into tree fruit pest management programs requires a basic knowledge of these insects' biology and behaviour (McNeil 1991). Knight *et al.* (1994) studied the temporal aspects of male and female activity for all four species. More detailed studies of these species' sexual behaviours have been reported for codling moth (see Howell 1991 for a review), orange tortrix (Knight 1996), and obliquebanded leafroller (Delisle 1992, 1995). This paper reports similar information on the temporal aspects of female calling, mating, and oviposition of PLR.

Crop protection using mating disruption for PLR may be difficult to achieve in orchards due to a number of biological factors that include the occurrence of high population densities in orchards and the potential for immigration from other orchards and alternative hosts (Brunner 1983, 1993). The behavioural ecology of PLR also likely limits the success of using sex pheromones for mating disruption. For example, moths are sexually active following emergence, call over a broad time period, can lay a large number of eggs following a single mating, resume calling after mating, and will remate. These behaviours of PLR, as well as similar behaviours reported for other tortricid pest species are highly adaptive and robust to ensure mating success. For example, as virgin *C. rosaceana* age they initiate calling earlier and call more frequently as they age (Delisle 1992; Fig. 1). The initiation and duration of sexual activity of many tortricids can be shifted in response to temperature fluctuations to increase mating success (reviewed in McNeil 1991). Low temperatures can shift the calling periodicity earlier and moths that normally call only during scotophase may initiate calling under normally inhibitory high light intensities (*C. rosaceana*: Delisle 1992; *C. pomonella*: Castrovillo and Cardé 1979).

The presence of conspecific sex pheromone can also affect moth behaviour. Weissling and Knight (1996) found that while the temporal patterns of calling and oviposition by *C. pomonella* were unaffected by the presence or absence of its sex pheromone, the frequency of calling by virgin females was significantly higher in codlemone-permeated air than clean air. A similar increase in calling in the presence of its own sex pheromone was reported with the tortricid, *Choristoneura fumiferana* (Clemens) (Palaniswamy and Seabrook 1985). Shifts in the calling periodicity in the presence of its own sex pheromone have been reported for *C. fumiferana* (Palaniswamy and Seabrook 1985) and the tea leafroller *Adoxophyes* sp. (Noguchi and Tamaki 1985).

These examples of the behavioural plasticity of some tortricids suggest that the advantage of employing mechanical 'smart' pheromone dispensers (Shorey *et al.* 1996) that could be turned on at a certain time in response to a specific light intensity threshold, or in relation to a specific temperature may be minor. Successful implementation of sex-pheromone-based mating disruption will occur in situations where constraints imposed by the pest's population dynamics, mating system, and management are minimized (Cardé and Minks 1995). Complete spatial and temporal flooding of the treated habitat with the selected semiochemical is most likely the best approach for maximizing mating disruption. Further studies of moth behaviour in the presence of their sex pheromones and antagonists, clarification of the mechanisms of mating disruption under different application systems, and a more complete knowledge of the role of minor sex pheromone components are three important avenues for future research.

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